CHIMERIC FILOVIRUS GLYCOPROTEIN

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INTRODUCTION

Marburg virus (MBGV) was first recognized in 1967, when an outbreak of hemorrhagic fever in humans occurred in Germany and Yugoslavia, after the importation of infected monkeys from Uganda (Martini and Siegert, 1971, *Marburg Virus Disease*. Berlin: Springer-Verlag; Smith et al., 1982, *Lancet* 1, 816-820). Thirty-one cases of MBGV hemorrhagic fever were identified that resulted in seven deaths. The filamentous morphology of the virus was later recognized to be characteristic, not only of additional MBGV isolates, but also of Ebola virus (EBOV) (Johnson et al., 1977, *J. Virol.* 71, 3031-3038; Smith et al., 1982, *Lancet* 1, 816-820; Pattyn et al., 1977, *Lancet* 1, 573-574). MBGV and EBOV are now known to be distinctly different lineages in the family Filoviridae, within the viral order Mononegavirales (Kiley et al., 1982, *Intervirology* 18, 24-32; Feldmann and Klenk, 1996, *Adv. Virus Res.* 47, 1-52).

Few natural outbreaks of MBGV and EBOV disease have been recognized, and all proved self-limiting, with no more than two cycles of human-to-human transmission. However, the actual risks posed by MBGV and EBOV to global health cannot be assessed because factors which restrict the virus to its unidentified ecological niche in eastern Africa, and those that limit its transmissibility, remain unknown (Feldmann and Klenk, 1996, supra). Concern about MBGV is further heightened by its known stability and infectivity in aerosol form (Belanov et al., 1996, *Vopr. Virusol.* 41, 32-34; Frolov and Gusev Iu, 1996, *Vopr. Virusol.* 41, 275-277). Thus, laboratory research on MBGV and EBOV is necessarily performed at the highest level of biocontainment. To minimize future risk, our primary interest has been the identification of appropriate antigens and vaccine strategies that can provide immunity to MBGV and EBOV.

The filovirus genome is composed of a non-segmented, negative sence, single stranded RNA of approximately 19 Kb. The open reading frames of the genome encode seven viral proteins: GP, the surface viral glycoprotein; NP, the major nucleocapsid protein; VP30, a minor nucleocapsid protein; VP35, a nonstructural protein; VP40 and VP24, internal membrane associated proteins; and L, the viral RNA-dependent RNA polymerase. In addition, Ebola virus encodes and 8th protein termed sGP (secreted glycoprotein). This protein is transcribed from the same open reading frame as full length GP. The function of sGP is unknown.

Vaccination with Marburg or Ebola GP protects from disease and death in rodent models of infection. MBGV GP delivered by Venezuelan equine encephalitis (VEE) virus replicon protected guinea pigs against infection by a homologous strain of Marburg virus (Musoke) (Hevey et al, 1998, Virology 251: 28-37). Similarly, vaccination with EBOV GP delivered by VEE replicon or DNA vaccination also elicited protective immunity in rodent models of Ebola virus infection (Pushko et al., 200, Vaccine 19, 142-153; VanderZanden et al., 1998, Virology 246, 134-144). However, when guinea pigs vaccinated with VEE replicons expressing MBGV (strain Musoke) GP were challenged with a heterlogous strain of MBGV (Ravn), complete protection was not observed, indicating that genetic variability among filoviruses may present an obstacle in obtaining a broadly cross reactive vaccine. Not surprisingly, vaccination with MBGV GP does not confer protection to challenge with Ebola virus, and vice versa. The GP proteins of MBGV Musoke and MBGV Ravn are 78% identical, compared to only 27% identity between MBGV Musoke and Ebola strain Zaire, indicating that genetic variability among filoviruses may present an obstacle in obtaining a broadly cross reactive vaccine.

Therefore, there is a need for an efficacious broadly cross-reactive vaccine able to protect against multiple filovirus strains.

SUMMARY OF THE INVENTION

The present invention satisfies the need discussed above. The present invention relates to a method and composition for use in inducing an immune response which is protective against infection with different filoviruses. More specifically, the present invention describes a single-component bivalent vaccine protective against both Ebola and Marburg viruses which is cost-effective and efficient to produce, develop and test. The present invention also embodies the production of similar vaccines, whether, bivalent, trivalent, or multivalent able to elicit a protective immune response to multiple filovirus agents in a single-component. Two strains of filoviruses were chosen, Marburg Musoke and Ebola Zaire which are the most divergent of all the filoviruses. If protection against both of these particular most-divergent strains is achieved in a single chimeric GP, chimeras made up of any other filovirus strains would be expected to also be cross-protective.

In order to elicit an immune response to both Marburg and Ebola, a chimeric glycoprotein was constructed using GP from each of the two viruses. Two forms of the glycoprotein are produced by transcription of the Ebola virus GP gene. Transcription and translation of the genomic copy of the GP gene results in a secreted, truncated form of the membrane-bound GP, termed sGP. The transmembrane GP protein is encoded in two open reading frames (ORFs) via a co-transcriptional editing mechanism whereby the viral polymerase inserts a non-templated adenosine residue at a specific editing site consisting of a run of seven adenosine residues (Volchkov et al, 1995, Virology 214, 421-430; Sanchez et al., 1996, J. Gen. Virol. 73, 347-357). Translation of the resulting mRNA has a frameshift at the editing site, such that sGP is identical to GP up to the editing site (aa295) but divergent thereafter, with a unique 69aa carboxy-terminus. MBGV, in contrast, encodes a single transmembrane GP protein using a single ORF (Will et al., 1993, J. Virol. 76, 1203-1210). The GP proteins of MBGV, (strain Musoke, 681aa, Genbank accession #Z12132 S55429, Feldmann et al., 1992, Virus Res. 24, 1-19) and EBOV (strain Zaire, strain Mayinga, 676aa, Genbank accession #U23187) are 27% identical and 39% homologous. Sequence differences between the two GPs are clustered in a hypervariable region spanning the middle third of the protein, while the N and C termini are more conserved. Both MBGV and EBOV GPs are highly glycosylated and are post-translationally cleaved by a host-cell furin-like protease into two disulfide-linked subunits, termed GP1 and GP2 (FIG. **1**) (Volchkov et al., 2000, Virology 286, 1-6; Volchkov et al., 1998, Proc. Natl. Acad. Sci. USA 95, 5762-5767; Becker et al., 1996, Virology 225, 145-155). The transmembrane anchor is located within the GP2 subunit, while the majority of the glycosylation sites are located within GP1 (Feldmann et al., 1999, Arch. Virol. 15, 159-169).

The purpose of our chimeric proteins was to construct a full-length GP (normally consisting of two subunits, GP1 and GP2) containing a subunit from EBOV with the other subunit from MBGV. There was no duplication or deletion of amino acid sequence from the heterologous virus GP in the portions that were cloned together, in order to preserve as much as